

FUNGI IN SWEDISH CONIFEROUS FOREST SOILS.

FUNGAL BIOMASS AND ACTIVITY

AND

MICROFUNGAL SPECIES COMPOSITION.

av

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AKADEMISK AVHANDLING

som för avläggande av filosofie doktorsexamen vid matematisk-naturvetenskapliga fakulteten vid Universitetet i Lund kommer att offentligens försvaras på Mikrobiologiska institutionen, hörsalen, Sölvegatan 21, Lund, tisdagen den 18 april 1978, kl 10.00.



ERRATA

ungi in Swedish coniferous forest soils. - Fungal bio-
mass and activity and microfungal species composition.
engt Söderström 1978

Page	line	Reads	Should read
Summary			
1	18	..pine forest	..pine forest soil
4	22	..Barron(1968).	..Barron (1972).
6	22	..& Williams 1977).	..& Williams 1971).
9	19	..1.1 g DW m ⁻² .	..1.5 g DW m ⁻² .
I. Soil microfungi in three Swedish coniferous forests			
7	22	..speceis..	..species..
1	3	<u>A. cf...</u>	<u>Acremonium cf...</u>
1	13	<u>Oididiodendron..</u>	<u>Oidiodendron..</u>
5	7	-most..	-highest..
7	24	.. <u>P. spinulosum</u> I,	.. <u>P. spinulosum</u> ,
II. Mycelial lengths and fungal biomasses in some Swedish coniferous forest soils, with special reference to a pine forest in Central Sweden.			
3	10	180-940 m g ⁻¹ DW	180-2100 m g ⁻¹ DW
3	25	1.1-17.7 mg DW	1.1-31.3 mg DW
3	27	146 g DW	141 g DW
3	28	102.6 g DW	97.6 g DW
6	20	..temperature of the year is..	..temperature is..
6	Table 2:2	B300-400 mm	B200-300 mm
6	Table 2:3	B300-400 mm	B200-300 mm
24	12	Merril	Merrill
28	15	decisions..	decision..
28	18	..dry weight..	..wet weight..
32	21	..confusion..	..conclusion..
40	21	,	;

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Lund 1978

This thesis is a summary of the following communications referred to in the text by Roman numerals:

- I. SÖDERSTRÖM B.E. 1975. Vertical distribution of micro-fungi in a spruce forest soil in the south of Sweden. Trans.Br.mycol.Soc. 65, 419-425.
- II. SÖDERSTRÖM B.E. and BAATH E. 1978. Soil microfungi in three Swedish coniferous forests. Holarctic Ecology 1 (in press).
- III. BAATH E. and SÖDERSTRÖM B.E. 1977. Mycelial lengths and fungal biomasses in some Swedish coniferous forest soils, with special reference to a pine forest in Central Sweden. Swedish Coniferous Forest Project. Uppsala. Technical Report 13. 45 pp.
- IV. SÖDERSTRÖM B.E. 1977. Vital staining of fungi in pure cultures and in soil with fluorescein diacetate. Soil Biol.Biochem. 9, 59-63.
- V. SÖDERSTRÖM B.E. 1978. Seasonal fluctuations of active fungal biomass in horizons of a podzolized pine forest in Central Sweden. Manuscript submitted to Soil Biol. Biochem.

1. INTRODUCTION

All organic material deposited on and in the soil will be decomposed and mineralized, mainly through the activities of fungi and bacteria. Considering their central position in the mineral cycles in nature, and thus also their invaluable role for the plant nutrition, the understanding of the activity, ecology and population dynamics of these organisms in their natural environment is remarkably sparse (Stotzky 1972).

The reports included in this thesis aim at giving some basic information on fungi in Swedish coniferous forest soils (I,II, III,V) and in addition a new method for the determination of metabolically active hyphae is described and its ecological application is pointed out (IV). The field studies were made at three sites: Kongalund, a planted spruce forest in Skåne, South Sweden, Nickobacken, a mixed coniferous forest in Dalarna, Central Sweden, and Ivantjärnsheden, a pine forest in Gästrikland, Central Sweden. At Nickobacken and Ivantjärnsheden the soils are well developed iron podzols with organic horizons consisting of a litter layer (A_{00} , L), a fermentation layer (A_{01} , F) and a humus layer (A_{02} , H). Below these is found an eluvial (bleached) horizon (A_2 , E) and an illuvial (enrichment) layer (B), both of somewhat varying thickness. At Kongalund the podzol is under development. The litter, fermentation and humus layers are found here too, but the mineral horizons are not typically podzolic and are designated A_1/A_2 and (B) respectively.

The results presented include microfungal species composition at all the sites, the distribution of the species in the soil horizons and the soil fungal biomass in the horizons. The biomass comprises all fungal mycelium in the soil, basidiomycete, mycorrhizal as well as microfungal, and was studied with two methods: one commonly used for the study of total biomass which was used at all the sites, and one developed for the study of metabolically active fungal biomass which was used at Ivantjärnsheden.

No similar studies have previously been made in Sweden, and although only three sites were studied, some of the results may also apply to Swedish coniferous forest soils in general.

2. MICROFUNGAL SPECIES COMPOSITION (I and II).

Soil microfungi were isolated from five different soil horizons at the three sites by use of the soil washing technique. This technique favours vegetative fungal structures at the expense of other propagules like spores and conidia (Parkinson & Williams 1961). Isolation medium was carboxymethylcellulose agar, which is a very non-selective medium.

Totally more than 4 000 isolations were made and 126 species were identified (II, Appendix).

Identification to generic level was mainly made by using the keys given by von Arx (1974) and Barron (1968). Identification to species level was obtained by using special literature. Many identifications were checked by the Centraalbureau voor Schimmelfcultures, (CBS), Baarn, Holland, and all species have been kept as references in a culture collection.

Pronounced differences in species composition between the soil horizons were obtained, while great similarities were found between corresponding horizons at the different sites. The micro-fungal flora may in fact show greater differences in two soil layers only a few cm apart than the flora in the same horizons at two sites hundreds of km apart. Thus, the podzolic soil horizons formed in Swedish coniferous forests seem to create environments suitable for a rather specific microfungal flora.

At all the sites the most frequently isolated microfungal genus was Mortierella Coemans, the mean isolation frequencies being 41.1, 29.7 and 37.4 % of the total number of isolates at Kongalund, Nickobacken and Ivantjärnsheden respectively. Altogether 20 species were identified and eight of these were found at all the sites. On the whole, the genus was found to be common in both organic and mineral soil layers, although different species dominated in the different horizons.

The genus Penicillium Link ex Fr. was also isolated in high frequencies: 13.8 - 19.6 % of the isolates at the three sites. This genus seemed to prefer soil layers with high organic matter content. In all, 19 different species were identified, but only one was found at all the sites. At Kongalund Trichoderma Pers. ex Fr. was also commonly isolated, but at the other two sites this genus was less frequent. Only the above-mentioned three genera exceeded 10 % of the isolates at any site.

All species which were found at two or three sites are listed in II, Table 3, and many of these can be regarded as common in Swedish coniferous forest soils.

The lists of species presented do not, of course, include all fungi present in the soil. The method used undoubtedly favours some microfungi, and a number of fungi, e.g. some basidiomycetes, are impossible to cultivate on the media used. Thus, the lists are not complete, but since the same isolation method was used at all the sites, the results are quite comparable. However, direct comparisons with studies made by other workers can only be made with difficulty. Not only the isolation technique influences the results but, for example, the sampling procedure and the identification accuracy are also of great importance. Thus no detailed comparisons are made with earlier studies, but generally the results reported here agree fairly well with results from coniferous forest soils in other countries (e.g. Kendrick 1963, Hayes 1965, Parkinson & Balasooriya 1967, Widden & Parkinson 1973).

3. TOTAL SOIL FUNGAL BIOMASS (III).

The total soil fungal biomass was estimated by a slightly modified agar-film technique (Jones & Mollison 1948). This method has been proved to be the most accurate and precise method for quantitative studies of fungi in soil (Nicholas & Parkinson 1967). It must be emphasized that the results obtained with the agar-film technique include both living and dead hyphae (Parkinson, Gray & Williams 1977).

At all the sites five horizons were studied, and at Ivantjärns-heden the mycelial lengths and fungal biomasses were determined by sampling regularly throughout one year. At the other two sites only solitary determinations were made. The fungal biomass was

calculated from the hyphal lengths by using the average cross section or the mean widths of the hyphae in each horizon.

Large amounts of fungal hyphae were found in all soils, although somewhat lower values were obtained at Kongalund. Most hyphae per g dry soil was always found in the A_{01} and A_{02} layers. Calculated per g loss on ignition, these differences were less pronounced. No obvious seasonal fluctuations in total fungal biomass were found.

At no site did the mean diameters of the hyphae exceed $3\text{ }\mu\text{m}$ in any horizon. However, the biomass in the organic layers was dominated by somewhat thicker hyphae than was the case in the mineral layers at Ivantjärnsheden, where the biomass mainly consisted of hyphae thinner than $3\text{ }\mu\text{m}$.

At Ivantjärnsheden the fungal biomass m^{-2} down to 30 cm below the surface of the B layer was 141 g DW, 70 % of which was detected in the mineral horizons. Calculations revealed that about 20 % of the total amount of nitrogen and phosphorus in the organic layers (A_{01} and A_{02}) was bound in fungal mycelium, mainly in cell walls. Thus, a substantial part of the potential plant nutrients in the soil is bound by the fungi and the rate of release from this nutrient pool is unknown.

Compared to earlier investigations in other countries (III, Table 2:8) the hyphal lengths at the sites studied were rather large, especially at the two sites in Central Sweden.

4. ACTIVE FUNGAL BIOMASS (IV and V).

The total fungal biomass determined with the agar-film technique consists of living as well as dead mycelium (Parkinson et al. 1971). The living part may be further subdivided into a dormant and a metabolically active fraction. The active portion of the biomass is the biologically and ecologically most interesting and important one. However, information about the living or active fractions of the fungal biomass is almost completely lacking, since only very recently methods have been available for the study of these (Parkinson et al. 1971, Frankland 1975, Flanagan & van Cleve 1977).

In order to enable studies of the active hyphae in the soil, a new method was developed (IV). This method makes use of a fluorogenic substance, fluorescein diacetate (FDA), which only fluoresces after enzymatic hydrolysis. Thus, if fungi are suspended in an FDA-containing solution the cells with active hydrolyzing enzymes will show a bright fluorescence. More than fifty different fungal species were tested in pure cultures for their staining ability with FDA, and all showed good or excellent staining properties of cells in actively growing stages. Determinations of the respiration and the staining ability after addition of a poison to actively growing cultures showed that FDA is a true vital stain, that stains only metabolically active fungal cells

The FDA-staining method was used for a more than two-year-long study of active mycelium in three horizons of the soil at Ivantjärnsheden (V). From the hyphal lengths recorded, the biomass was calculated by using the mean hyphal diameters, the density and dry weight of mycelium given in III.

The amount of active hyphae showed recurrent peaks in autumn and spring. In unfrozen soil, the peaks seemed to correlate well with increased soil moisture contents, indicating that this factor is of major importance for fungal activity in nature. The temperature did not seem to directly influence the amount of active biomass, except for freezing and thawing of the soil when the active biomass decreased and increased respectively. Increase in active fungal biomass was also noted during the winter when the soil temperature was below 0°C .

In all horizons the amount of FDA-active mycelium was very small compared to the total amount. In A_{01}/A_{02} , A_2 and B the mean lengths of active hyphae were only 2.4, 4.3 and 2.6 % respectively of the mean total lengths. It is highly probable that the proportions of active mycelium change with the season, but this has not been studied in detail.

The FDA-active biomass per m^2 was equally distributed between the A_{01}/A_{02} horizon (5 cm) and the $A_2 + B_{0-10 \text{ cm}}$ (15 cm) layers. The highest value obtained was 2.4 g DW m^{-2} and the mean value of all determinations was 1.1 g DW m^{-2} . The corresponding total biomass was 83.6 g DW m^{-2} (III).

For many years it has been supposed that in temperate regions an enhanced soil fungal activity takes place during spring and autumn (Burgess 1958), and due to this the biomass could be expected to increase during these periods. A number of workers have reported that their results show tendencies strengthening this assumption (e.g. Nicholas, Parkinson & Burgess 1965, Latter, Cragg & Heal 1967, Hanssen & Goksdøyr 1975). However, they have studied the

biomass changes only for one year or less, and for a periodicity of this kind to be definitely demonstrated, the changes should be observed during at least two successive years. The only study found to range over more than one year is the one by Nagel-de Boois & Jansen (1971), who were unable to demonstrate any recurrency in the registered biomass peaks, as determined with the agar-film technique. That clear seasonal fluctuations could be demonstrated in the present study (V) is probably due to the fact that it is the first long-term study presented, where a technique has been used that selectively distinguishes the active biomass. Thus, the technique is much more sensitive to changes in the growing biomass than are methods that result in estimations of the total biomass.

Although the seasonal periodicity in active fungal biomass was studied at only one site, the results probably also apply to other soils, since the changes in the biomass mostly seemed to be an effect, directly or indirectly, of the gross climatic factors. It could thus be expected that the same pattern of periodicity exists in most coniferous forest soils in Central Sweden.

5. CONCLUDING REMARKS

In a podzol, the soil horizons are not only morphologically and chemically different, but they also seem to differ biologically. It has been shown here that quite diverse microfungal populations dwell in the different soil horizons. It has also been demonstrated that the amount of hyphae and, for example, the hyphal diameters, differ considerably between the horizons. The factors that cause

these differences are, however, unknown. Parkinson & Balasooriya (1967) suggested that pH is a factor that may strongly influence the vertical distribution of fungal species, but a number of other factors may also be of importance (Burgess 1958). In the present study, the pH does not seem to be of major importance since great changes in the populations were found between soil layers with only minor changes in pH. Further studies are needed to explain the biological differences between the horizons.

The amount of active fungal biomass may be used for calculations of the relative importance of fungi in the energy turnover in the soil. To illustrate this some preliminary calculations will be given. The respiration rate of FDA-active fungal biomass and the Q_{10} relation of the respiration have been estimated in laboratory experiments (E. Bååth & B. Söderström, unpublished data). By using these results together with the biomass fluctuations (V, Fig 2) and the soil temperature during the year, the fungal respiration has been estimated to $110 \text{ g C m}^{-2} \text{ year}^{-1}$. The total above- and below-ground litter formation at the site is $441 \text{ g DW m}^{-2} \text{ year}^{-1}$ (Staaf & Berg 1977), which corresponds to about $220 \text{ g C m}^{-2} \text{ year}^{-1}$. Carbon added to the soil by root exudation is not included in this figure. It must, however, be stressed that these calculations are preliminary, and some of the conversion factors used may be corrected later.

The relation between the biomasses and the different fungal groups in the soil is quite unknown. We have no idea how much of the measured biomasses that are made up of for example the microfungi, the basidiomycetes or the mycorrhiza-forming fungi.

This is a question of great importance and studies aiming at a more detailed knowledge of the soil fungal biomass, activity and function are needed in the future.

6. ACKNOWLEDGEMENTS

I am grateful to Professor Börje Norén, Uppsala, who initiated my work and who has supported me in every way. Dr. Walter Gams, Baarn, Holland, and Mr. Erland Bååth, Lund, have given me invaluable help.

Dr. Birgit Hertz and Professor Claes Weibull, Lund, have been of much help. I am greatly indebted to Mrs. Gunilla Wernerheim for her skilful technical assistance. All help from colleagues and friends at the Department of Microbial Ecology, Department of Microbiology, Lund, and the Swedish Coniferous Forest Project, Uppsala, is also gratefully acknowledged.

The main part of this work was carried out within the Swedish Coniferous Forest Project supported by the Swedish Natural Research Council.

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